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Next Meeting of the Scientific Advisory Committee for Alternative Toxicological Methods (SACATM), Bethesda, 10-11 March 2004

Comments on Test Chemicals selected for the ICCVAM-ECVAM Cytotoxicity Validation Study

Dear Dr. Stokes, dear Bill

recently you shared with us the list of chemicals that have been selected as test chemicals in the ICCVAM-ECVAM Cytotoxicity Validation study currently under way. ZEBET was asked to comment on that list. The list contained detailed new information on multiple LD_{50} values per each test chemical, gathered from various literature sources. It came along with additional documents on acceptance and exclusion criteria for *in vivo* LD_{50} studies, as well as calculations for each chemical of the LD_{50} variability derived from the multiple LD_{50} values. Because the laboratories are testing these chemicals already, the expected input at this stage was most probably a comment on quality and acceptability of the *in vivo* LD_{50} data rather than on the chemical selection itself.

However, our analysis of the chemical selection raised serious concern that the chemical selection itself is so unbalanced that the main goal of the study (verification of Willi Halle's linrear RC prediction model) cannot be achieved. **With these test chemicals, the study outcome can only be a falsification of the RC prediction model**. Please find on the following two pages a detailed description of our concern.

Because it would be too detailed for the SACATM meeting, and in order not to loose track from our main concern, we will send later under separate cover other suggestions of tiny corrections and additions (e.g. new IC_{50} values from Halle's new RC3 for some of the selected chemicals).

We do hope that you will find time at the next SACATM meeting to discuss (and hopefully destroy) our concerns.

With our best regards
Sincerely yours

Manfred Liebsch, Willi Halle, Horst Spielmann and Elke Genschow
ZEBET at the BfR

ZEBET Comments on Chemical Selection for the ICCVAM-ECVAM Cytotoxicity Validation Study based on the following ICCVAM documents [ChemListAug03-alpha.XLS] and [ReferenceLD50sAug03.XLS]

Fifty nine (59) chemicals from Willi Halle's Registers of Cytotoxicity 1 and 2 (RC-1,2) are contained in the selection of test chemicals for the ICCVAM-ECVAM Cytotoxicity Validation Study. Of these 59 chemicals, 21 chemicals (36%) are outliers of the linear regression Prediction Model (PM) in terms of falling out of the acceptance boundaries of ± log 5.

This is about 10% more outliers than the incidence observed in all parts of the RC, including the new RC3 which has just recently been finalised. **The high percentage of outliers could be regarded an acceptable stressing of the robustness of Halle's PM if not 19 of the 21 chemicals (90%) were below the lower boundary (= false negative), and only 2 chemicals (10%) were above the upper boundary (= false positive)**. With this unbalanced bias in the selected test chemicals, it is impossible to verify the RC PM. In contrast, a falsification of the PM, resulting in a much steeper regression function will be the outcome of the study.

Figure 1 depicts the linear regression function of RC1,2 Prediction Model, data of the 347 chemicals and the ± log 5 acceptance boundaries as published in NIH Publication 01-4500. The 59 chemicals of the RC-1,2 selected for the ICCVAM-ECVAM Cytotoxicity Validation Study are marked **orange**. Already from that figure, an eye-ball guess results in a much steeper regression for these data. **Figure 2** shows the outcome of the calculation of new "ICCVAM regression function" (dashed line) based on the 59 selected RC chemicals.

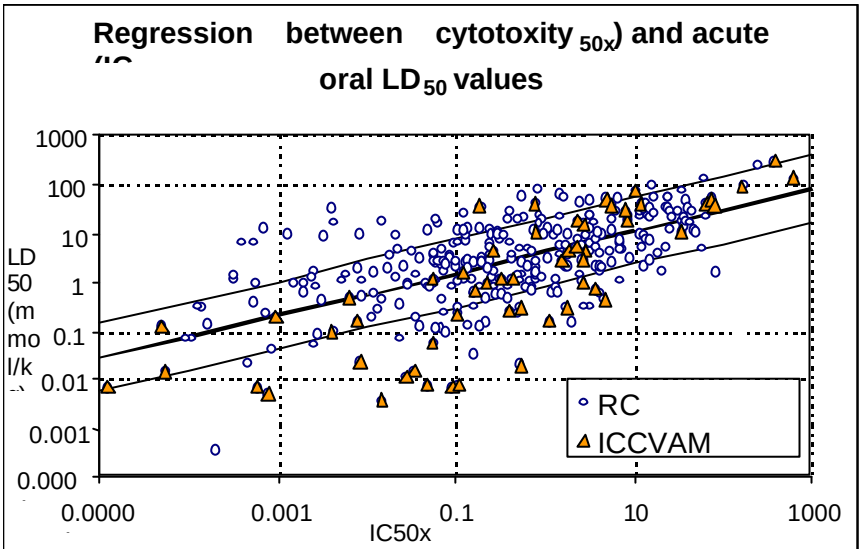


Figure 1

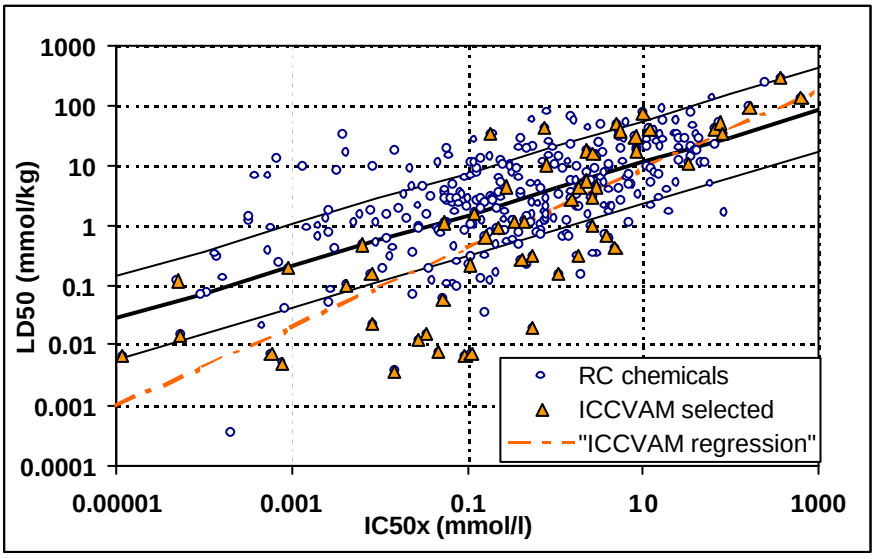
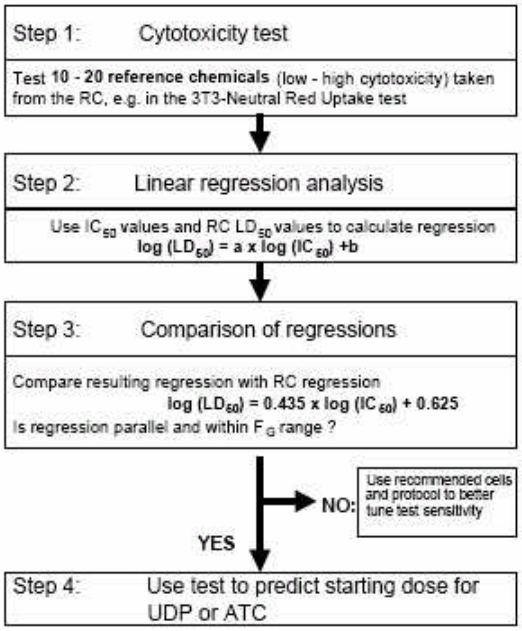


Figure 2

It is our understanding that in the ICCVAM-ECVAM Cytotoxicity Validation Study hard interlaboratory cytotoxicity data under GLP are generated in the 3T3-NRU and NHK-NRU assays to check if the RC regression (based on the IC_{50x} approach from various literature data) can be confirmed with data derived from a controlled experimental study. If this were the case, the assay(s), together with the RC prediction model could be recommended for regulatory use as predictors of the *in vivo* starting dose or limit dose tests in one of the new acute oral toxicity test methods UDP, FDP, or ATC.

Thus, the objectives of the ICCVAM-ECVAM Cytotoxicity Validation Study are comparable to the objectives of a single lab validation of any in-house basal cytotoxicity assay as described in the ICCVAM Guidance Document NIH Publication 01-4500 (see below cut-outs from this document).

Based on the 59 RC chemicals selected for the ICCVAM-ECVAM Cytotoxicity Validation Study the new regression would have the formula **$\log (LD_{50}) = 0.291 \times \log (IC_{50}) + 0.660$**. It would therefore neither be parallel to the RC regression line, nor would it be within the acceptance boundaries. As a consequence, a laboratory would have to follow the "NO" option between step 3 and 4 of the Figure given below.



Procedure for evaluating a cytotoxicity test for tiered *in vitro*/*in vivo* testing for acute oral toxicity testing (slightly modified from Spielmann et al., 1999).

Cut out from

NIH Publication 01-4500

If the regression line obtained with the candidate cytotoxicity test parallels the RC regression and is within the ± log 5 interval, then the test is considered suitable to generate IC_{50} data to use with the RC regression for estimating starting doses (Figure 2, Step 4). The rationale for using the RC regression rather than the regression from the candidate cytotoxicity test is that the RC regression is based on data from 347 chemicals, while the candidate regression is based on data from only 10-20 chemicals. To predict an LD_{50}

Finally, we want to share with you an interesting part of our *in vivo* data analysis:

At the ICCVAM *In Vitro* Workshop 2000 it was questioned whether the one NIOSH LD_{50} value per chemical was in fact represetative enough to counterbalance the IC_{50x} backed by the geometric mean of multiple cytotoxicity data.

We have plotted the geometric means of the multiple LD_{50} values from the new ICCVAM *in vivo* data inventory against the one NIOSH LD_{50} value listed in the RC: with a few exemptions, a perfect 1:1 correlation

(see **Figure 3** on the right →)